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Research Article

Hematological and Biochemical Indices of a Local Rabbit Does Population Fed Diet Incorporated with Jujube Pulp (*Ziziphus Lotus*)

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Abstract
Rabbit feed in Algeria is still based exclusively on commercial pelleted diets, but the recent use of non-conventional ingredients in feed formulation is becoming more frequent, and several studies were carried out on the incorporation of local raw materials in rabbit feed worldwide. However, studies related to the incorporation of Jujube are non-existent. In this context; this experimental trial assessed the impact of Jujube pulp on local populations' rabbits' hematological and biochemical parameters during three physiological stages: non-pregnancy, pregnancy, and lactation. 72 rabbits were divided into 18 groups of 04 rabbits each one, three groups of non-pregnant females fed 100 % commercial pelleted diet, three groups of non-pregnant females fed 50 % commercial pelleted diet with 50 % Jujube, three groups of pregnant females fed 100 % commercial pelleted diet, three groups of pregnant females fed 50 % commercial pelleted diet with 50 % Jujube, three groups of lactating females fed 100 % commercial pelleted diet, and three groups of lactating females fed 50 % commercial pelleted diet with 50 % Jujube. The levels of white blood cells (WBC), hemoglobin concentration (Hb), lymphocytes (L %), mean corpuscular volume (MCV) and Platelets (PLT) recorded in pregnant and lactating rabbits fed a diet incorporating Jujube were greater than those found in females fed 100 % commercial pelleted diet. The results revealed also that 79 % of the variations in ALP, 79 % in creatinine and 57 % in Tb expressed were related to variations in diet and physiological stage. The hematological and biochemical profile of the experimental animals indicated that Jujube is nutritionally appropriate without any deleterious effect on the female rabbits.

Keywords: Hematological profile, incorporation, Jujube, local rabbit does, physiological stage.

Introduction

Rabbit farming has been a longstanding practice in Algeria. It is widespread across the country, present in nearly all over Algeria with a notable concentration in disadvantaged areas, arid or desert regions, and on the outskirts of small urban centers. It provides an important protein intake. Similarly, it has the major advantage to enhance many lots of unusable products and by-products. Several years ago, there was an attempt to encourage the adoption of modern intensive techniques for rabbit breeding. Nevertheless,

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these breeding methods have not fully embraced the technical advancements made in this field. Moreover, the rabbit has frequently been overlooked in development initiatives due to a lack of understanding of its real contribution to the coverage of animal protein needs of the populations [1,2]. Family rabbit farming persists, particularly in rural communities, where it serves as a supplementary income and a valuable source of protein. When conducted on a small scale, this type of production enables each family to generate meat for home consumption. However, when undertaken on a larger scale, it has the potential to generate substantial income and profits for the entire family, knowing that the farm is an activity that is still high in most cases [3] In addition, the development of rabbit farming in Algeria is typically characterized by the use of small local populations for breeding [4,5,6], fed on a diet exclusively based on a commercial pellet with lower nutritional quality [7], constituting around 60 to 70 % of the total production cost [8]. Indeed, increasing the use of nutrients is crucial to the profitability and sustainability of rabbit production [9], hence the need to seek other alternative and locally available food sources. This exploration is justified by the fact that rabbits have the ability to convert forage and other agricultural products into highly nutritious meat, but also because the recent use of non-conventional ingredients in feed formulation is becoming increasingly common [10].In fact, several research studies were conducted in this field [11,12,13,14,15]. Using Jujube as a dietary supplement for animals is a promising application under investigation [16]; its availability, relatively low cost, and health benefits make it an attractive component for animal nutrition [17]. However, To the best of our knowledge, studies focusing on the use of Jujube in rabbits' diets are non-existent.This fruit belongs to the species *Ziziphus lotus*, also known as Berber Jujube or wild Jujube tree, commonly recognized in Algeria as "Nbeg." The Jujube is one of the oldest cultivated fruit trees in the world and the most significant species within the cosmopolitan Rhamnaceae family in terms of economic, ecological, and social importance [18]. It is also considered as one of the five most nutritious and healthiest fruits in ancient Chinese medicine [19]. This spiny and climbing shrub forms a bush that reaches a height of 1 to 2 meters. The very small leaves are alternate, oval or elliptical, more or less pubescent underneath, and are highly palatable to livestock [20]. As research on Jujube has grown, scientists have discovered numerous beneficial nutrients in this fruit, including carbohydrates, minerals, vitamins, sugars, and amino acids; consequently, Jujube is a highly regarded nutritious food worldwide [21,22].

Considered as a reliable mean for health assessment and nutritional status evaluation [23], hematological and biochemical parameters led scientists to investigate the effects of various ingredients on animal hematology and serum biochemistry. These studies showed that foods from different sources and their inclusion levels in diets influence physiological processes in organisms [24,25]. In this context, this study was conducted, aiming to determine the effect of the incorporation of Jujube pulp on common hematological and biochemical plasma parameters in local rabbit does population during non-pregnancy, pregnancy, and lactation stages.

Materials and methods

Animals and diets

The study was conducted in a rabbit farm in the region of Chlef, a city located in the northwestern part of Algeria, characterized by a semi-arid Mediterranean climate. It involved 72 female rabbits from local populations, with an average weight of 2000 ± 100 grams and aged between 5 to 6 months. The females were randomly divided into 18 experimental groups (n = 4 per group), based on three physiological stages (non-pregnant, pregnant, and lactating) and two diets (commercial diet and Jujube pulp diet). Jujube fruit was previously washed, dried, and peeled before being finely ground. The rabbits underwent a 15-day adaptation period, after which 36 females were introduced to males for mating, pregnancy was assessed through palpation. The females were divided as follows:

- Three groups of non-pregnant females (NPFC), three groups of pregnant females (PFC) and three groups of lactating (LFC) females, fed exclusively with commercial pellets,
- Three groups of non-pregnant females (NPFCJ), three groups of pregnant females (PFCJ) and three groups of lactating females (LFCJ), receiving a mixed ration of 50 % commercial pellets and 50 % Jujube.

Breeding and blood sampling

The female rabbits were housed in large cages in a disinfected space. Each female was identified individually using livestock spray and was vaccinated against various parasites, and viral infections. During the experiment, the animals were maintained under conditions of 25°C temperature, 75 % relative humidity, and 12-hour lighting duration per day. The rabbit does were fed commercial diet mainly composed with alfalfa, corn, wheat bran, soybean meal, soybean oil, calcium, phosphate, salt, as well as vitamins A, E,

D3, and copper, it contained 15 % crude protein, 2.5 % crude fat, 10 % ash, and 12 % crude fiber. The females received also Jujube pulp containing 4.27 % crude protein, 1.37 % crude fat, 5.8 % crude fiber, and 3.25 % ash. Fresh drinking water was freely available [26].

Regarding the blood samples, they were taken early in the morning from fasting females. Since cardiac puncture is not recommended for companion and farm animals, samples were taken using a flexible catheter by marginal ear vein. The blood samples used for hematological analyses, which included white blood cells count (WBC), red blood cells count (RBC), hemoglobin concentration (Hb), hematocrit value (HCT), lymphocytes percentage (L%), mean corpuscular volume (MCV), neutrophils percentage (N%), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets count (PLT), were collected into tubes containing EDTA and delivered immediately to the laboratory and promptly assayed. For biochemical analyses, additional blood samples collected in sterile tubes without anticoagulant were centrifuged at 3000 rpm for 10 minutes; the serum was then separated and stored at -20°C until analysis, to evaluate the contents of glucose (Glc), urea, creatinine, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (Tb), and alkaline phosphatase levels (ALP) [27]. The analyses were performed using automated analyzers from the Mindray and Beckman Coulter brands. The conditions of rearing, handling and using of the animals were approved by the Scientific Council of Laboratory of Natural Bio-Resources, at the Faculty of Nature and Life Sciences in University Hassiba Benbouali of Chlef in Algeria, and under the supervision of a veterinary clinician, with respect of the suitable standards for the welfare of the animals during the research, and adherence to ethical standards with rabbits.

Statistical analysis

The data collected were subject to descriptive statistical analysis using Excel, while analysis of variance (ANOVA) and multivariate analysis (PCA) were performed using a trial version of Xlstat 2022.1 [28].

Results

Hematological parameters

The results indicated that comparison of means showed significant to highly significant differences between the different females (Table 1). The WBC exhibited highly significant differences ($P < 0.0001$), the highest WBC values were observed in LFCJ, NPFCJ, and PFCJ, while the lowest were recorded by PFC and LFC. In terms of RBC, the differences were significant ($P < 0.05$), the highest counts were measured in NPFC, while the lowest were recorded by NPFCJ. *Regarding Hb, the* differences were highly significant ($P < 0.01$), PFC and LFCJ expressed the highest Hb concentrations, whereas the lowest were measured in PFCJ. The differences in L% percentages were highly significant ($P < 0.0001$), as well as the MCV ($P < 0.01$) in LFCJ, but the lowest values were respectively observed in LFC and NPFC. PLT exhibited highly significant differences ($P < 0.001$) in PFCJ and LFCJ compared to NPFC. Highest values of N% ($P < 0.0001$), MCH ($P < 0.001$), and MCHC ($P < 0.01$) were recorded in LFC. While, HCT was not statistically affected ($P > 0.05$) by the incorporation of Jujube.

Table 1. Hematological parameters of female rabbits fed commercial pellets and Jujube pulp

	WBC $10^3/mm^3$	RBC $10^6/mm^3$	Hb g/dl	HCT %	L %	MCV mm^3	N %	MCH pg	MCH C %	PLT $10^3/mm^3$
NPFC	6.24^{b}	6.36^{a}	9.83^{bc}	31.3^{a}	62.6^{ab}	57.6^{b}	24.7^{b}	18.5^{b}	33^{b}	309^{c}
NPFCJ	9.62^{a}	4.93^{b}	11^{abc}	33.3^{a}	61.3^{ab}	68.1^{a}	62.1^{a}	19.4^{b}	34.2^{b}	532^{ab}
PFC	3.56^{c}	6.05^{a}	12.7^{a}	37.5^{a}	37.7^{b}	64.9^{ab}	54.7^{a}	22.3^{a}	34.4^{b}	354^{bc}
PFCJ	9.08^{a}	5.26^{a}	9.71^{c}	30.8^{a}	42.8^{b}	61.4^{ab}	53^{a}	20.1^{ab}	33.7^{b}	650^{a}
LFC	3.20^{c}	5.49^{a}	12^{abc}	37.7^{a}	36.9^{b}	67^{a}	67^{a}	22.4^{a}	37^{a}	352^{c}
LFCJ	9.96^{a}	6.15^{a}	12.4^{ab}	32.2^{a}	81.7^{a}	68.4^{a}	62^{a}	20.5^{ab}	33.6^{b}	633.6^{a}
R^2	0.92	0.36	0.49	0.30	0.63	0.50	0.66	0.60	0.55	0.74
***P*-value**	< 0.0001	0.0473	0.0045	0.1078	0.0001	0.0032	< 0.0001	0.0003	0.0011	< 0.0001

Biochemical parameters

Significant to highly significant differences in biochemical parameters of the different rabbit does were also shown (Table 2). In terms of Glc, highly significant differences ($P < 0.01$) were observed; variations in diet associated to physiological stage explained 51% of the variations related to this parameter. NPFCJ, PFCJ, and LFCJ recorded the highest levels of Glc compared to the other females. Regarding urea, significant differences ($P < 0.05$) were expressed in relation to variations in diet and physiological stage, which explain 39% of the fluctuations in this parameter. This biochemical indicator exhibited the same trend as Glc, with higher concentrations shown by the females fed on Jujube during the three physiological stages. Furthermore, highly significant differences ($P < 0.001$) were observed in creatinine, ALP and Tb. 79 % of the variations in ALP, 79 % in creatinine and 57 % in Tb recorded were related to variations in diet and physiological stage. However, total cholesterol, triglycerides, AST and ALT were not significantly different ($P > 0.05$).

Table 2. Biochemical parameters of female rabbits fed commercial pellets and Jujube pulp

	Glc g/L	Urea g/L	Creatinine mg/L	Total cholesterol g/L	Triglycerides g/L	AST IU/L	ALT IU/L	Tb µmol/L	ALP IU/L
NPFC	1.36^{ab}	0.38^{ab}	7.78^{cd}	1.40^{a}	1.40^{a}	52^{a}	47.5^{a}	3.99^{bc}	19.2^{c}
NPFCJ	2.01^{a}	1.08^{a}	11.9^{ab}	0.80^{a}	1.77^{a}	63.6^{a}	56.4^{a}	4.40^{ab}	47^{ab}
PFC	1.31^{b}	0.30^{ab}	10.1^{bc}	1.10^{a}	1.41^{a}	76.5^{a}	63.8^{a}	3.71^{c}	44.1^{b}
PFCJ	1.97^{ab}	0.61^{ab}	11.7^{ab}	0.60^{a}	1.83^{a}	51.0^{a}	44.0^{a}	4.27^{abc}	73.4^{a}
LFC	1.40^{ab}	0.24^{b}	7.17^{d}	0.64^{a}	1.52^{a}	45.8^{a}	56.2^{a}	4.38^{ab}	74.6^{a}
LFCJ	1.94^{ab}	0.62^{ab}	10.1^{a}	0.63^{a}	1.83^{a}	53.6^{a}	48.2^{a}	4.71^{a}	70.7^{ab}
R^2	0.51	0.39	0.76	0.35	0.11	0.26	0.2	0.57	0.79
***P*-value**	0.003	0.03	< 0.0001	0.056	0.697	0.179	0.382	0.0006	<0.0001

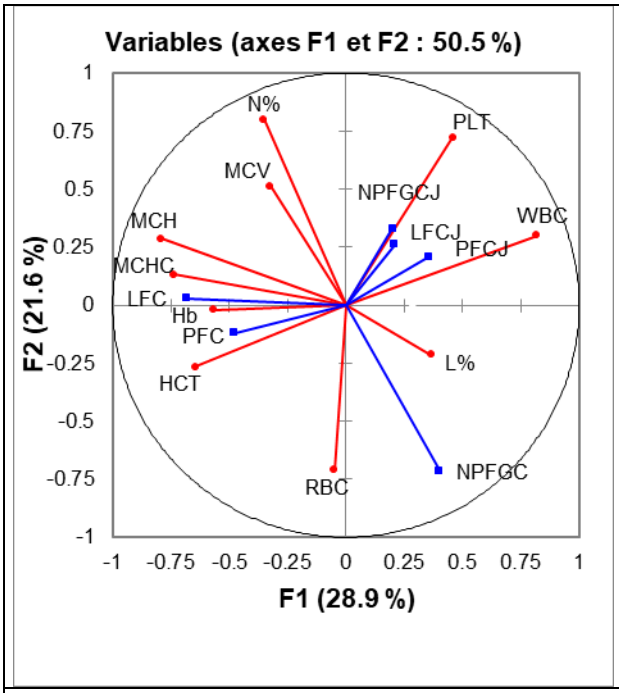
Discussion

The study revealed that the experimental diet had significant effect on some hematological parameters of the experimental rabbits. Considered as an approximate indicator of an animal's immune status [29], WBC content in the experimental females fed on Jujube was better. In addition, the WBC values recorded in pregnant females fed on Jujube were higher than those reported by Dabbou et al. [30] ($6.22\times10^3/mm^3$) in pregnant Grimaud rabbit does supplemented with *Echinacea* extract. Also, RBC values obtained in the pregnant females receiving Jujube pulp were in line with earlier observations of the same author ($5.62 \times10^6/mm^3$) in the same experimental females. *The lowest Hb concentrations showed by PFCJ* (9,71 *g/dl)* were near the normal ranges *reported by* Flecknell [31] (10 – 15 *g/dl). This rate was may be due to* anaemia which often occurs during pregnancy. *Nevertheless, the levels of Hb reported by Henry et al [10] (8.92 to 10.21 g/dl) in cross-bred rabbits (New Zealand X Chinchilla) fed fresh and wilted papaya leaves, and Unung et al [25] (10.4 ± 0.21 to 10.2 ± 0.26 g/dl) in Californian white rabbits supplemented with 13% and 18% crude protein were lower than those recorded in NPFCJ.*The increase i*n Hb content* observed in some females fed Jujube, may be supported by several available *reports confirming the beneficial effects of Jujube polysaccharides in preventing anaemia [22].* The highest values of L% expressed by LFCJ can be justified by the works of Zhao et al. [32], who proposed the structures of rhamnogalacturonan and its side chains in the Ju-B-2 polysaccharide, which contribute to the immune response. Additionally, Cai et al. [33], in their research on immune activity, demonstrated that Jujube could promote the proliferation of spleen lymphocytes. The PLT in PFCJ has greatly exceeded those obtained by Dabbou et al. [27] ($157\times10^3/mm^3$) in pregnant Grimaud rabbit does supplemented with *Echinacea* extract. The MCH recorded were consistent with the levels mentioned by Amao et al. [34] in rabbits fed on diet incorporated with 15 % of African Star Apple Seed Meal.

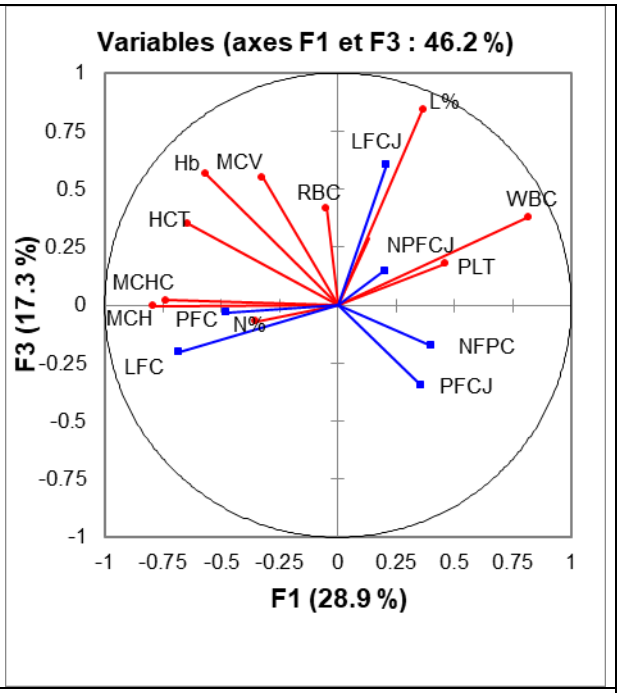
Compared to the work of Onu and Aja [35], who evaluated hematological indices of rabbits fed ginger and garlic supplemented diets, the high concentrations of WBC, Hb, lymphocytes, MCV, and PLT recorded in this study in PFCJ and LFCJ indicated that Jujube pulp likely contains modulatory factors that can stimulate greater blood production in these rabbits compared to those fed with pellets. This hypothesis is further supported by Zheng et al. [36], who found that

Jujube flavonoids stimulate erythropoietin expression, promoting blood production, it also suggest the beneficial effect of the incorporation of Jujube on the rabbits' immune system. Leukocytes counts are of significant clinical importance as they contribute to the body's immune defense [37], and WBC elevation is frequently a reliable sign of improved immunity [38]. According to Hao et al. [39], substituting 10 or 15 % of corn with Jujube powder in the diet of Taihang chickens reduced stress, enhanced growth and development of immune organs, improved resistance to pathogens, and increased survival rates.

The principal component analysis revealed that the first three axes explained 67.8 % of the information (Figure 1). The first axis, explaining 28.9 % of the variability, is strongly correlated to WBC and to a lesser degree to PLT. The high values of WBC and PLT mainly characterize NPFCJ, LFCJ, and PFCJ. Conversely, this axis is negatively correlated to MCHC, MCH, HCT, and Hb, which predominantly characterise LFC and PFC. The second axis, explaining 21.6 % of the variability, is strongly correlated to N%, PLT, and MCV. NPFCJ seem to be more influenced by these three variables. This second axis is negatively correlated to RBC characterising NPFC. The third axis, explaining 17.3 % of the variability, is mainly positively correlated to L% and to a lesser degree to Hb and MCV, LFCJ are most distinctly characterized by these hematological variables.



Projection of variables and different rabbit does in the plan 1x2



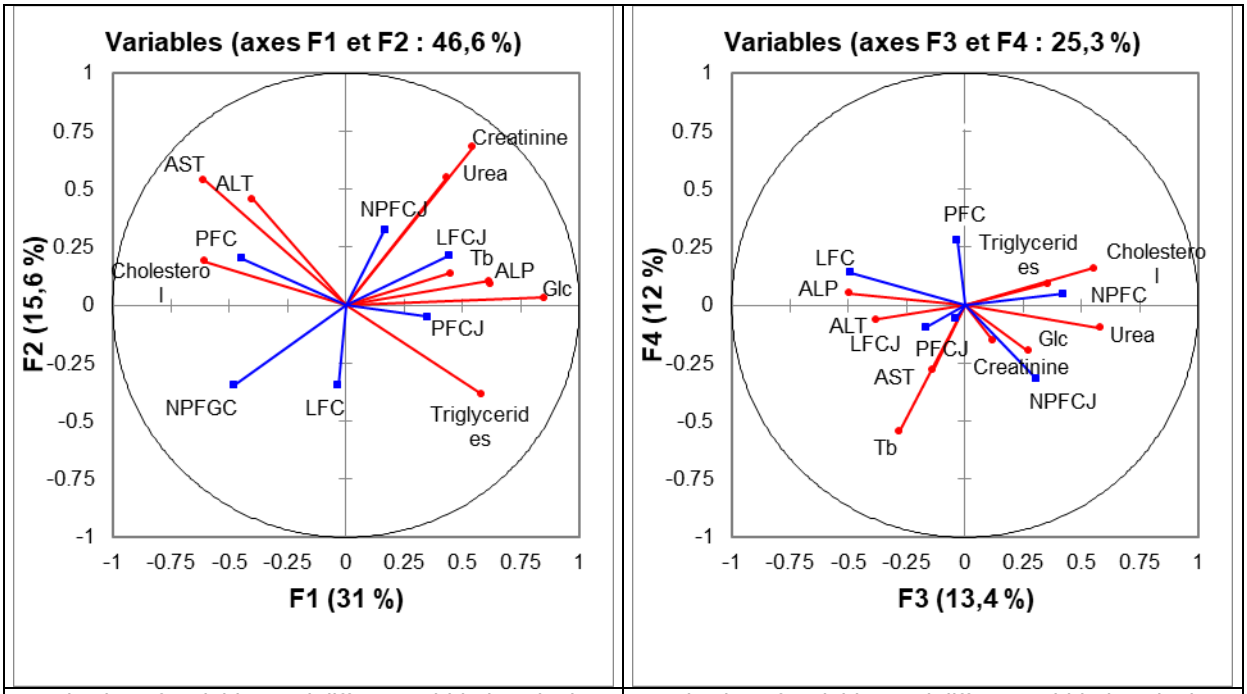
Projection of variables and different rabbit does in the plan 1x3

Figure 1. Projection of the different parameters (haematological variables and rabbit does) on the three axes of the PCA

The analysis of serum biochemical parameters showed that rabbit does fed on Jujube during all the physiological stages exhibited the highest levels of Glc, in comparison with those fed on pelleted diet. The levels of blood glucose recorded in NPFCJ were markedly different from those obtained by Yao Konan et al. [40] (0.81 to 0.94 g/L) in rabbits fed with various palatable leafy vegetables. The high level of this parameter is due to the predominance of glucose in Jujube pulp [41,42,43], and it is well known that under heat stress, glucose levels rise [44]. Furthermore, Özkan et al. [27] reported that the variations in several parameters, such as stress, blood collection methods, housing conditions, are thought to be the cause of elevated glucose levels in rabbits. The levels of urea in NPFCJ were notably lower than those recorded by Onu and Aja [36] (5.99 to 6.45 g/L) in rabbit does fed on diet enriched with garlic and ginger, which frankly indicate good hepatic function and comfort [41] in our rabbit does fed on Jujube. The levels of creatinine in NPFCJ were higher than those reported by Aboh et al. [45] (7.0 to 8.25 mg/L) in

rabbits fed Moringa oleifera leaves pellets. According to Njidda and Isidahomen [46], good creatinine values suggest the absence of muscle tissue catabolism. The Tb in NPFCJ corroborate those in the physiological range of healthy rabbits mentioned in MediRabbit [47] (3.4 to 8.5 µmol/L). ALP is also within the reference range of blood parameters in rabbits established by Leineweber et al. [48] (9.05 to 94.68 IU/L), and agree with the values found by Amao et al. [35] (56.1 to 76.5 IU/L) in rabbits fed a graded level of African apple seed meal. In addition, there were no significant differences among diets and physiological stages for total cholesterol, triglycerides, AST and ALT, their levels are in agreement with the findings in some research works involving plant incorporation [49,50,35], thus justifying the hepatoprotective effect of Jujube already reported by Selim et al. [51]. Indeed, Liu et al. [52] demonstrated that protocatechuic acid, vanillic acid, p-coumaric acid, catechol and p-hydroxybenzoic acid are the main compounds responsible for this effect. These findings can be supported by several pharmacological studies revealing the wide range of health benefits associated to this fruit [53,18,54,19,44,55].

According to the principal component analysis the first four axes explained 71.9 % of the information (Figure 2). The first axis, which alone explained 31 % of the information, is strongly positively correlated to Glc, ALP, triglycerides and creatinine, but is negatively correlated to AST and total cholesterol. Analysis of this axis also showed that females fed on diet incorporated with Jujube have high levels of Glc, ALP, triglycerides and creatinine, but low levels of AST and total cholesterol, whereas the opposite was observed in females on a strictly commercial diet. The second axis, with 15.6 % of the information explained, is positively correlated to the variables: creatinine, urea and AST. In terms of diet, this axis is positively correlated to NPFCJ, whereas it is negatively correlated to triglycerides, associated to NPFC and LFC. The third axis, with 13.4 % of variability explained, is mainly positively correlated to urea and total cholesterol and to NPFC, but it is negatively correlated to ALP related to LFC. The fourth axis, with 12 % of variability explained, is negatively correlated to Tb, mainly associated to NPFCJ.



Projection of variables and different rabbit does in the plan 1x2

Projection of variables and different rabbit does in the plan 3x4

Fig. 2. Projection of the different parameters (biochemical variables and rabbit does) on the four axes of the PCA

Finally, the knowledge of reference values for hematological and biochemical parameters in rabbits provides researchers and veterinary doctors with information they can use in their research and clinical practice concerning animal health [56]. The hematological and biochemical

components for the different indices measured in this study are within the physiological range reported in healthy rabbits according to MediRabbit [47]. However, the difference in results obtained compared with other studies carried out on effects of different incorporated diets in rabbits, can be attributed to certain factors influencing these parameters, in particular the physiological status of gestation and lactation [57], but also the species, breed, sex, age, diet tested and biological rhythm specific to each animal [58].

Conclusions

This investigation showed that the blood indicators were within the normal range, supporting the well-being and physiological state of the local rabbit does. The hematological and biochemical profile of the experimental rabbits indicates that Jujube is nutritionally suitable without any deleterious effects on the animals. The results also revealed the potential usefulness of Jujube pulp as a supplement in the rabbit diet, as well as its effectiveness in enhancing hematological and biochemical characteristics. It can be considered as a promising supplement for use in commercial rabbit production. However, it would be of further interest to test the effect of Jujube on the growth of the youngs as well as on the nutritional quality of the rabbit meat obtained. Additional research is also necessary to document the benefits of this dietary incorporation in male rabbits and under various environmental conditions.

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